INDUCTION OF GENE AMPLIFICATION IN DZHUNGARIAN HAMSTER CELLS BY SOME CHEMICAL CARCINOGENS

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It has been shown in recent years that one way by which mammalian somatic cells can undergo genetic variation is through amplification (i.e., an increase in the number of copies) of single genes. The phenomenon of gene amplification evidently plays an important role in carcinogenesis. In a high proportion of cases of certain human malignant neoplasms (neuroblastomas, small-cell carcinoma of the lungs, etc.) amplification of certain cellular protooncogens (N-myc, c-myc, etc. respectively) is found [6, 7, 9]. Other evidence of the possible participation of gene amplification in tumor development is given by the results of a study of the effect of various chemical compounds on the probability of development of this phenomenon. Certain agents capable of inducing or inhibiting DNA synthesis, including the carcinogenic compounds 20-methylcholanthrene (MCh), 7,12-dimethylbenz(a)anthracene (DMBA), and 12,0-tetradecanoylphorbol-13-acetate (TPA), have been shown to be inducers of gene amplification [2, 6, 9, 10].

This paper describes an attempt to determine which classes of chemical carcinogens increase the probability of development of gene amplification.

METHODS

The following substances were used: TPA, 4-O-methyl-TPA, mezereine (all from Sigma, USA), ethylmethanesulfonate (EMS), nitrosomethylurea (NMU), and aflatoxin B_1 (all from Serva, West Germany), DMBA and Tween-80 (both from Ferak, West Germany), MCh (from Fluka, Switzerland), Colchicine (CCh, from Merck, West Germany), and methotrexate (MTX, from Lederle, USA).

Experiments were carried out on Dzhungarian hamster cells of line DM-15 [1]. The cells were cultured in medium RPMI 1640 containing 10% bovine serum and 100 U/ml of monomycin.

The effect of the substances on the probability of appearance of gene amplification was judged on the basis of their action on the frequency of appearance of variants resistant to CCh and MTX in the population. Resistance to each of these preparations arises as a rule as a result of amplification of certain regions of DNA [3, 4, 5, 9]. Investigation in both test systems made it possible to determine with reasonable certainty whether the carcinogen tested induces gene amplification.

The cell populations were treated with the test compounds before seeding on selective medium (0.05-0.075 μg/ml of CCh or 0.06-0.075 μg/ml of MTX under conditions causing death of the 20-80% of the cells. The method of determining the frequency of cell colonies resistant to CCh and MTX was described by the writers previously [2].

RESULTS

The effect of nine compounds belonging to different classes of carcinogens was investigated. They included various promotors of tumor growth (TPA, mezereine, Tween-80), the polycyclic hydrocarbons DMBA and MCh, the alkylating compounds EMS and NMU, and also aflatoxin B_1. The nonpromoting TPA analog, 4-O-methyl-TPA, was used as the negative control.

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TABLE 1. Effect of Mezereine on Frequency of Appearance of Cell Colonies Resistant to CCh and MTX

Dose of mezereine, μg/ml	Selective agent (SA)	Dose of SA, μg/ml	Frequency of colonies	Increase in number of colonies compared with control (number of times)
0	CCh	0,060	$7,5 \cdot 10^{-5}$	1,0
		0,075	$<9,2 \cdot 10^{-6}$	1,0
	MTX	0,060	$1,3 \cdot 10^{-3}$	1,0
		0,075	$6,9 \cdot 10^{-4}$	1,0
0,03	CCh	0,060	$4,2 \cdot 10^{-4}$	5,6
		0,060	$6,4 \cdot 10^{-3}$	5,0
	MTX	0,075	$4,4 \cdot 10^{-3}$	6,4
0,3	CCh	0,060	$8,8 \cdot 10^{-4}$	11,7
		0,075	$1,9 \cdot 10^{-5}$	>2,1
	MTX	0,060	$1,3 \cdot 10^{-2}$	10,0
		0,075	$8,1 \cdot 10^{-3}$	11,7

Legend. The frequency of the colonies was determined allowing for efficiency of colony formation of cells treated and not treated with mezereine in respective medium. The increase in the number of colonies is the ratio of frequency of colonies after treatment with mezereine to the frequency in the control population.

TABLE 2. Effect of Some Chemical Carcinogens on Frequency of Appearance of Cells Resistant to CCh and MTX

Compound	Conditions of treatment	Increase in number of colonies	
		CCh-resistent	MTX-resistent
TPA	0,01—0,1 μg/ml, 72 h	7,8—44,9	9,6—128,0
4-O-methyl-TPA	0,01—0,1 μg/ml, 72 h	0,4—3,4	1,0—2,5
Mezereine	0,03—0,3 μg/ml, 72 h	5,6—56,3	5,0—11,7
Tween-80	0,01—0,1 mg/ml, 72 h	3,0—6,2	1,2—22,5
DMBA	1,5—5,0 g/ml, 72 h	21,6—25,2	10,1—12,5
MCh	3,0—10,0 g/ml, 72 h	12,4—128,0	28,7—72,8
Aflatoxin B_1	1,0—5,0 g/ml, 72 h	2,5—35,0	8,2—82,6
EMS	0,3 mg/ml, 24 h	0,2—1,3	1,8—2,0
NMU	0,15—0,3 mg/ml, 3 h	0,1—3,8	0,5—0,9

Legend. The increase in the number of colonies is shown as the ratio of the frequency of colonies after treatment with the substances to the frequency in the control population.

All compounds were tested in 3-5 experiments. In each experiment different concentrations of the carcinogen and of the selective agents were used. The results of one experiment to study the effect of mezereine on the frequency of appearance of CCh- and MTX-resistant cells are given in Table 1. After treatment with mezereine the proportion of both CCh- and MTX-resistant cells was increased by 5-11 times in the population; the frequency of the colonies increased, moreover, with an increase in the dose of mezereine.

Data on the effect of all the compounds studied on the frequency of appearance of CCh- and MTX-resistant cells are summarized in Table 2. Treatment with tumor growth promotors (TPA, mezereine, Tween-80), and also with the majority of carcinogens with a marked action, both inducing and promoting (DMBA, MCh, aflatoxin B_1), increased the proportion of both CCh- and MTX-resistant cells in the population. The compounds listed above evidently have ity to induce gene amplification.

Meanwhile 4-O-methyl-TPA, which does not possess full promoting activity, and the alkylating compounds EMS and NMU, with more marked inducing activity, were evidently not powerful inducers of gene amplification. Ability to induce gene amplification may perhaps correlate with the promotor activity of carcinogens.

Recent investigations have shown that promotion is a multistage process which can be divided into at least two stages [8]. The possibility cannot be ruled out that the ability of carcinogens to induce gene amplification correlates to some degree with their ability to induce the second stage of promotion. Evidence in support of this view is given by the fact that TPA (a strong inducer of both stages of promotion [8]) and mezereine (a weak inducer of the first, but powerful inducer of the second stage of promotion) increase the probability of gene amplification, whereas 4-O-methyl-TPA (an inducer of the first, but not of the second stage of promotion [8]) does not induce gene amplification. Further investigations must show to what extent this hypothesis is true.

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